

Antimicrobial activity of root canal filling materials

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Abstract

The antimicrobial activity of filling materials was studied to improve the quality of treatment of complicated caries in vitro. Resorcin-formalin cement Resodont ("Latus", Ukraine) - № 1; Endocort zinc oxide-eugenol cement ("Latus", Ukraine) - No. 2; materials based on zinc oxide eugenol cement with dexamethasone: Endomethasone ivory (Septodont, France) - No. 3 and Endofil ("Produit Dentaires SA", Switzerland) - No. 4. To assess the antimicrobial activity of the studied materials, test microorganism strains were used: Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 6783, Candida albicans ATCC 885-653.

As a result of the study, it was established that filling materials have different antimicrobial activity. Resodont has the greatest antimicrobial activity on the test culture. Endomethason is active in relation to the test cultures studied, inferior to Resodont in its antimicrobial properties. Endocort and Endofil have less antimicrobial activity than Resodont and Endomethason, but have approximately equal growth retardation rates. It should be noted that with respect to Staphylococcus aureus, Endocort was found to be almost 1.5 times more active than Endofil.

The conducted studies confirm that the materials for filling of root canals by Resodont and Endocort (Latus, Ukraine) have a pronounced antibacterial effect. They are competitive with foreign sealers and are the materials of choice, having a significantly lower cost.

Key words: endodontic microbiology; root canal treatment; antimicrobial activity of sealers

Introduction

Despite accumulated experience knowledge and modern abilities in endodontic, the percentage of failed treatment of complicated caries in Ukraine is quite high, main causes of unsatisfactory endodontic treatment are disorders of standards of irrigation and filling of root canals. Often dentists do not consider the features of filling materials for obturation [1].

In endodontic practice there are number of root canal obturation techniques, each of them has its own advantages and disadvantages. Nowadays, most popular method among endodontists, is obturation of root canals with gutta-percha and sealer [2, 3].

According to many prominent researchers' perfect sealer should not irritate the periodontal tissues, densely obturate root canals both in lateral and vertical directions, not let the sealer shrink in root canals, it should also have antibacterial effect, be hydrophobic, biocompatible, nontoxic and radiopaque. it should not affect the tooth color and at the same time should be able to polymerase quickly, should have good adhesive feature to the dentine and gutta-percha [4, 5].

For final obturation of root canals, different filling materials are used: based on zinc oxide eugenol (with different therapeutic additives) and epoxy resin, and resorcinol-formaldehyde and etc. [6].

Zinc oxide-eugenol pastes represent cements modified for endodontic treatment [7, 8, 9]. Advantages of this sealers are connected to prolonged antimicrobial effect which is provided with releasing of eugenol. Using zinc-oxide-eugenol cements as sealers immediately after the filling of root canals provides the absence of microbial insertion in periodontium, which mostly depends on quality of obturation of root canals and not on the effect of antibacterial paste. Positive features of zinc oxide-eugenol cements are anti-inflammatory and pain relieving effects and the ability to use it in relatively dry root canal.

This material is pliable, easy to insert and extract from root canal, has satisfactory adhesive feature to canal walls and does not shrink. Despite advantages there are disadvantages too, for example it can destroy the polymerization of composites, has a strong cytotoxic effect on the culture of the fibroblast. Inhibits the function of macrophage [10; 11]. For enhancing the positive qualities of the materials in this group, the

additional components are introduced: corticosteroids, thymol, paraformaldehyde, etc. Their cytotoxic and carcinogenic effects are proved.

Analysis of modern domestic and foreign literature shows that there is still no filling material for the root canals of the teeth, which would have the universal antimicrobial properties and could satisfy all the requirements of dentists in the treatment of complicated caries.

The studies of many authors have shown that composition of the root canals microflora depends on the nature of inflammatory process. Most often in the contents of the root canals are anaerobic microorganisms. The key element in the success of caries complications treatment is the choice of filling materials [11].

Currently, there is a large amount of zinc – oxide eugenol cement for the root canal obturation on the market, the cost of which depends on the manufacturer. There was set a task before us - to study the antimicrobial properties of some obturation materials, in order to improve the quality of treatment of complicated caries.

The purpose of the study: a comparison of the antimicrobial activity of in-vitro 4 materials for the root canal filling.

Materials and methods

The antimicrobial activity of filling materials was studied. Samples: Resorcin-formalin cement Resodont (“Latus”, Ukraine) - № 1; Zinc oxide-eugenol cement Endocort (“Latus”, Ukraine) - № 2; Two materials based on the zinc oxide-eugenol cement with dexamethasone: Endomethasoneivory (“Septodont”, France) - № 3 and Endofil («ProduitDentairesSA», Switzerland) - №4.

According to the WHO recommendations, to evaluate the antimicrobial activity of the studied materials the microorganisms test-strains were used: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 6783, *Candida albicans* ATCC 885-653 [12]. The microbial load was 10^7 microbial cells per 1 ml of environment and was established according to the mcfarland standard. There were used 18-24 hours of microorganism cultures, while the *C. Albicans* culture was preliminarily grown on Saburo environment with 2% glucose solution, and the cultures of *S. Aureus* and *E. Faecalis* - on Mueller-Hinton agar (HI media, India).

The antimicrobial activity of filling materials was determined by the method of “wells” (a method of diffusion into agar) with determination of diameters in the zones of growth retardation of microorganisms [13, 14]. This method is based on the ability of the active ingredient of the drug to diffuse into the agar on which the seeding of the test culture is performed. Determination of the antibacterial properties of materials was carried out on two layers of the dense nutrient environment poured into Petri dishes. In the lower layer, the non-seeded environment of Muller-Hinton was used: the melted nutrient environment was poured into Petri dishes in the amount of 10 ml; after agar hardening, the sterile stainless steel cylinders 10 mm high and 9 mm in diameter were applied to it. 4 cylinders were placed in one cup. In parallel, the melted nutrient environments were

poured in 13.5 ml into test tubes, where, after cooling the agar to 40-45°C, 1.5 ml of suspension of microorganisms was added. The top layer was thoroughly mixed and poured: it was poured around the cylinders obtained from 15 ml of environment, seeded with the appropriate microorganism. After solidification of the top layer of agar with steriletweezers, the cylinders were removed and in the formed holes the study drug was put, prepared in accordance with the instructions of manufacturers.

The plates were kept for 30 min at room temperature and then the seedings were incubated in a thermostat at 37°C for 18–24 h. Observations and calculations were carried out for 3 days at zones of growth retardation around “wells” (in mm), including the diameter of the “well” itself. The medicinal substance of the drug diffuses into the agar, forming around the “well” a zone of inhibition of the growth of microorganism’s sensitive to it, clearly standing out against the background of continuous growth. If the zones of oppression had an oval shape, then, in such cases, the largest and smallest diameters of the zone were measured and the average value was calculated, which was taken as an indicator.

Evaluation of antibacterial properties was carried out according to the following criteria:

Absence of the zones of growth retardation of microorganisms around the hole, as well as diameters of growth inhibition zones up to 10 mm indicate that the microorganisms are not sensitive to the sample introduced into the well, the drug was classified as inactive;

The zones of growth retardation of microorganisms with the diameter of 10-15 mm indicate a low sensitivity of the culture, a moderately active sample;

The zones of growth retardation of microorganisms with the diameter more than 15 mm are regarded as an indicator of sensitivity of the microorganism to the studied samples; the preparation was classified as an active agent.

For the reliability of the obtained results, the study was repeated three times. The data obtained during the study were subjected to statistical processing. The reliability of the identified differences of the studied parameters was evaluated using the Mann-Whitney test for independent samples [15].

The microbiological studies were conducted at the base of Department of Clinical Immunology and Microbiology of the Kharkov Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine.

The research results and their discussion

The study showed that the filling materials have different antimicrobial activity, which depends on the type of microorganism and the chemical composition of the material (Table 1.).

Study of the antimicrobial activity of various materials for root canal filling in relation to reference microorganism strains

Test-culture	Zones of growth retardation of microorganisms around the hole with filling material, mm			
	№1	№2	№3	№4
<i>Candida albicans</i> ATCC 885/653	50,2±2,0*	20,0*	40,0*	22,0*
<i>Staphylococcus aureus</i> ATCC 25923	50,0	34,1	50,4	22,1
<i>Enterococcus faecalis</i> ATCC 6783	35,1*	12,0	25,0*	14,2

Note: * - secondary culture growth; №1 - Resodont («Latus», Ukraine); №2 - Endocort («Latus», Ukraine); №3 - Endomethasone ivory («Septodont», France); №4 - Endofil («Produit Dentaires SA», Switzerland).

Table 1

The greatest bacteriostatic effect on *Candida albicans* was expressed in Resodent, where the zones of growth retardation of microorganisms was - 50,2 mm and Endomethasone - with the zone of growth retardation - 40.0 mm. While Endofil, the zone of growth retardation was within 22.0 mm, and at Endocort - 20.0 mm. These results show that all materials actively suppress the growth of *Candida albicans*.

But at the same time, we can state that after curing the material, we detected a secondary growth of the culture, which indicates activity of the material only until the moment of complete polymerization as a result of the direct activity of phenolic compounds released during curing (Fig. 1.)

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Figure 1: Accounting of growth inhibition zones *C. albicans* ATCC 885-653 (method of "wells").

Filling material : №1 - Resodent («Latus», Ukraine); №2 - Endocort («Latus», Ukraine); №3 - Endomethasone ivory («Septodont», France); №4 - Endofil («Produit Dentaires SA», Switzerland).

The significant changes are observed in the study of growth retardation of *St. aureus*. Resodent and Endomethasone have expressed bacteriostatic activity – 50,4 and 50,0 mm growth retardation zones, respectively. Endocort also actively suppresses the growth of this microorganism, which is 34.1 mm. The growth retardation indicators

in Endofil - is 22.1 mm, which indicates its lowest activity to *St. aureus* compared to other materials. The results obtained characterize that all the materials as active agents' relative to *St. Aureus* (Figure 2). In addition, the given materials were also active after their polymerization, as evidenced by the data on the absence of secondary growth of a culture of staphylococcus.



Figure 2: Accounting of growth inhibition zones *S. aureus* ATCC 25923 (method of "wells"). Filling material: №1 - Resodent («Latus», Ukraine); №2 - Endocort («Latus», Ukraine); №3 - Endomethasone ivory («Septodont», France); №4 - Endofil («Produit Dentaires SA», Switzerland).

Growth of *E. faecalis* inhibits Resodent - the zone of growth retardation is 35.1 mm and Endomethasone - 25 mm, but at the same time we observe appearance of the secondary growth around the holes with the indicated materials (Fig. 3). Endofil had a moderate activity to the reference strain

of *E. faecalis* (with a growth retardation zone of 14.2 mm) and the lowest activity in this study had Endocort - 12 mm, but at the same time, Endofil and Endocort did not have the secondary growth of enterococcus culture.



Figure 2: Accounting of growth inhibition zones *E. faecalis* ATCC 6783 (method of "wells"). Filling material : №1 - Resodont («Latus», Ukraine); №2 - Endocort («Latus», Ukraine); №3 - Endomethasone ivory («Septodont», France); №4 - Endofil («Produit Dentaires SA», Switzerland).

In this way, the filling materials have different antimicrobial activity. Resodont has the greatest antimicrobial activity on the test-culture. Endomethasone is active in relation to the examined test-cultures, inferior to Resodont in its antimicrobial properties. Endocort and Endofil have less antimicrobial activity than Resodont and Endomethasone, but have approximately equal growth retardation rates. It should be noted that relative to *Staphylococcus aureus* Endocort was almost 1.5 times more active than Endofil.

The conducted studies confirm that the materials for filling root canals Resodont and Endocort ("Latus", Ukraine) have an expressed antibacterial effect. They are competitive with the foreign sealers and are the materials of choice, having a significantly lower cost. The materials of the company "Latus" (Ukraine) are available in ergonomic and aesthetic packaging. All this allows us to recommend Resodont and Endocort for use for endodontic treatment.

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